QMP and QAPP for Land-based testing of the BioViolet™ BWMS

Project ID: BioViolet™

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February 2011

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Introduction & Background

Kwang San Co., Ltd. Republic of Korea is seeking the Type Approval from the Korea Ministry of Land, Transport and Maritime Affairs (MLTM) for its proprietary UV system – brand name BioVioletTM.

Such approval is being sought in accordance with the Provisional Regulation for Type Approval of Ballast Water Management System' by the Ministry of Land, Transport and Maritime Affairs and (PR. No. 2011-342, Annex 4 and Annex 6) and *Guidelines for Approval of Ballast Water Management Systems* (G8) and *Procedure for Approval of Ballast Water Management Systems that Make Active Substances* (G9) as adopted by Res. MEPC 174(58) (2008) and 169(57) (2008) of the IMO, respectively.

Part 2, Section 2.1.2 of the G8 and Section 4.2.4 of the G9 require that the testing process for the ballast water management system should include both a Quality Management Plan (QMP) - which addresses the overall quality management policies and structures of the test organization(s) (including sub-contractors and outside laboratories); and a Quality Assurance Project Plan (QAPP) - which provides detailed quality assurance arrangements for the actual testing procedures.

Accordingly, Kwang San Co., Ltd. and its primary testing body for the Approvals – the Korea Marine Equipment Research Institute (KOMERI) in Busan – have developed QMP and QAPP for the Approvals of the BioVioletTM; and both are presented in this document.

As the G8 and the G9 do not provide any guideline as to the structure, format and content of the required QMP and QAPP (except to refer to generic international standards), and as such guideline is not available from IMO, Kwang San Co., Ltd. and KOMERI have adopted the following as the standard for their QAPP:

- US EPA Requirements for Quality Assurance Project Plans (EPA QA/R-5)
- US EPA Guidance for Quality Assurance Project Plans (EPA QA/G-5)
- KOMERI KOLAS Manual, Procedure and direction documents, 2008
- KTR KOLAS Manual, Procedure and direction documents, 2009

Quality Statement

Full Title of the quality assurance document:	QMP and QAPP for Land-based test for Bioviolet™
Project ID:	Bioviolet™
Organizations to which the quality assurance document applies:	1) Kwang San Co., Ltd., Busan, Republic of Korea 2) Korea Marine Equipment Research Institute (KOMERI), Busan, Republic of Korea
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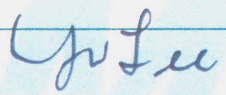
Position:	Chief Technology Officer
Signature:	
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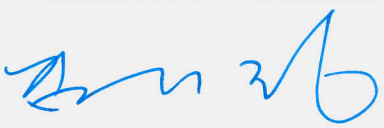
Signature:	
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Quality Management Plan (QMP)

1 PROJECT ORGANIZATION

1.1 Kwang San Co., Ltd.

Quality management system of Kwang San Co., Ltd. provides the overall QMP for all of research, development, and testing activities related to BioViolet™. As a designer, developer, manufacturer and supplier of industrial products in the international market, quality management, quality assurance and quality control are integral components.
Kwang San Co., Ltd. maintains a corporate quality management system certified by certificates such as ISO 9001:14001 standards by KFQ. A copy of the current certificate is attached to Appendix 3.5.

The certified quality management system of Kwang San Co., Ltd. is as follows:

- ISO 9001 : 2008
- OHSAS 18001 : 2007
- ISO 14001 : 2004

1.2 KOMERI

All laboratories at the Korea Marine Equipment Research Institute (KOMERI) comply with the Korea Laboratory Accreditation Scheme (KOLAS – www.kolas.go.kr) system for laboratory and research organizations as outlined in Korean national regulations, to ensure the consistency and reliability of laboratory testing results.

All testing and analytical procedures and equipment at KOMERI are certified by the KOLAS to KS ISO/IEC 17025 standard. The certification number for KOMERI is KT-190 and a copy of the current KOLAS accreditation is attached as Appendix 3.5.2.

KOMERI is accredited by the Ministry of Land, Transport and Maritime Affairs as a Type Approval Test Organization in accordance with the Interim Regulation for Type Approval of Ballast Water Management System and Res. MEPC 125(53) of the IMO. A copy of the current certificate is attached as Appendix 3.5.2.

The KOLAS accreditation of KOMERI provides the overall QMP (ISO/IEC 17025) and QAPP for all of its testing activities relating to the BioVioletTM.

Scope of Certificate for Quality Management System of KOMERI is as follows:

.1Test facility management
.2 Field sampling
.3 Sample transit
.4 Sample preservations
.5 Laboratory and Field Analyses
.6 Measurement and Data acquisition

2 PROJECT MANAGEMENT

2.1 Project responsibilities

All activities for the achieving of the Type Approvals of the BioVioletTM must be controlled under KOMERI's supervision (Figure 1).

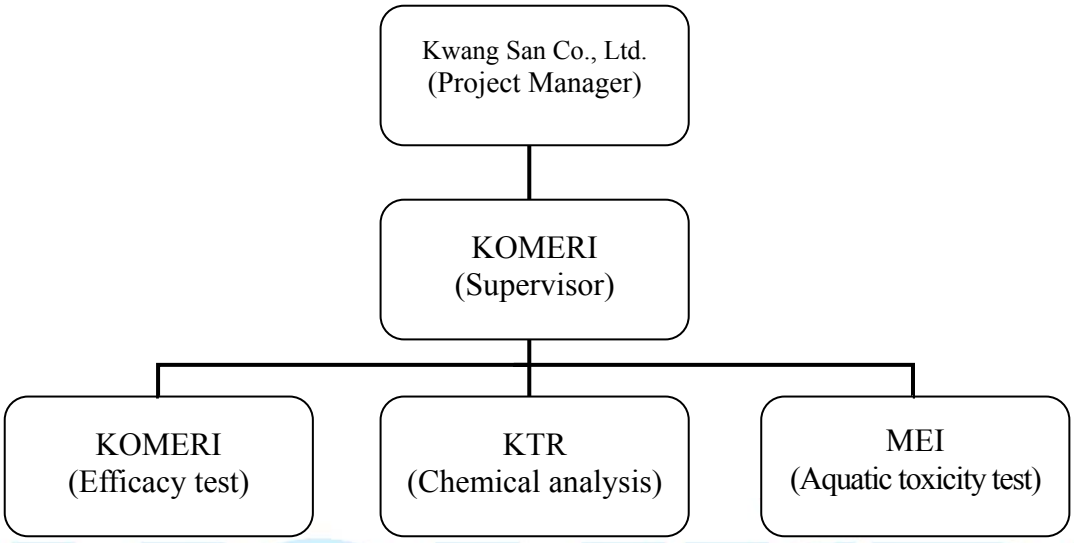


Figure 1. Overall project management arrangement for the Type Approvals of the BioVioletTM.

Table 1. Distribution list

Organization	Task	Quality system
KOMERI	**Efficacy test** - Organism viability (≥ 50 μm) - Organism viability (≥ 10-50 μm) - *E. coli*/Coliform - Intestinal Enterococci - Heterotrophic bacteria - Toxicogenic *V. cholera*e (O1, O139)	- ISO/IEC 17025 - Certified testing institute for Type Approval by the MLTM, Korea (Land-based and shipboard test)
KTR	**Qualitative and quantitative chemical analysis** - Active Substance - Relevant Chemical - Other component	ISO/IEC 17025
MEI[a]	**Aquatic organism toxicity test** - Algae growth inhibition test - Acute toxicity test - Chronic toxicity test	-

[a] All procedure of MEI is complied with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17).

The overall Project Organization for testing in accordance with the G8 and G9 are summarized in Figure 2 and Table 2.

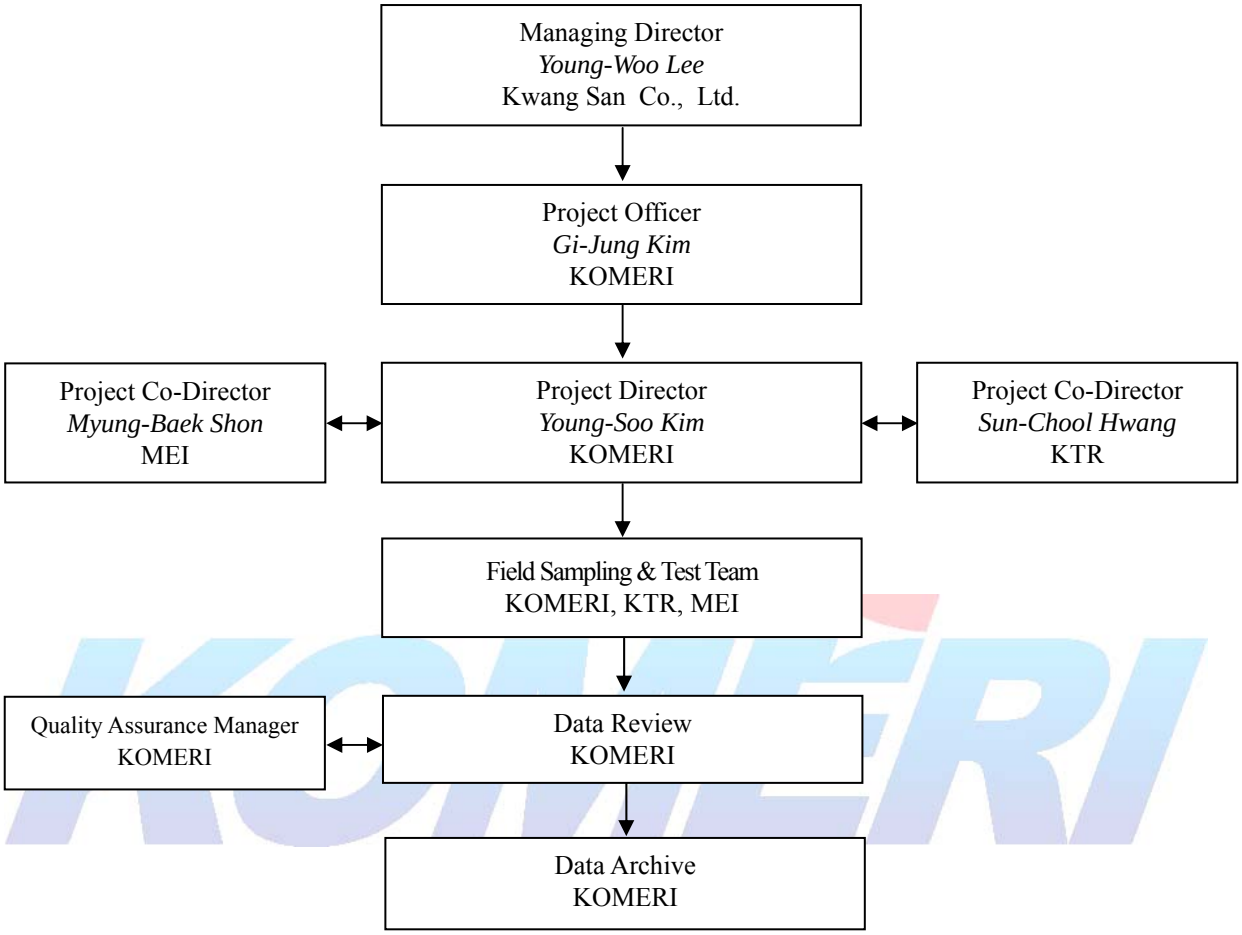


Figure 2. Organizational chart outlining the relationship among parties

Table 2. Task responsibilities in the BioViolet™

Organization	Name	Responsibility
KOMERI	Gi-Jung Kim	Project Officer, President
	Young-Soo Kim	Project Director
	Jae-Uk Kang	Quality Assurance Manager
	Jun-Hak Lee	Test Facility Manager / Laboratory technician - Bacteria
	Jun-Hyuk Yang	Archive Personnel / Laboratory technician - ≥ 50 μm organism
	Hyung-Min Park	Field sampling manager/Laboratory technician -≥ 10-50 μm organism
	Eun-Ju Seong	Laboratory technician - ≥ 50 μm organism
	Chul-Hoi Hoe	Laboratory technician

		- ≥ 50 μm organism
	Jeong-Kyeong Park	Laboratory technician - ≥ 10-50 μm organism
	Seon Yeon Park	Laboratory technician - ≥ 10- 50 μm organism
	Eun Lee	Laboratory technician - ≥ 10- 50 μm organism
	Soo-Yeon Im	Laboratory technician - Bacteria
	Bo-ram Lee	Laboratory technician - Bacteria
KTR	Won-Tae Choi	Project Co-Officer
	Sung-Uk Lee	Project Co-Director
	Ji-Hyun Lee	Laboratory technician (Study director) - VOCs/THMs
	Young-Keun Im	Field Sampling Manager Laboratory technician - TRO/FRO - O_3, ClO_2, Bromide, ClO_3, Density
	Sun-Chool Hwang	Laboratory technician - Bromate - AOX - Halogenated phenols
	Jun-Ho Park	Laboratory technician - HANs
	Jin-Hoon Do	Laboratory technician - HAAs - DOC/POC
MEI	Min Ho Son	Project Co-Officer
	Myung-Baek Shon	Project Co-Director (Study director) Laboratory manager - Aquatic toxicity on Algae
	Jin-Hee Kim	Project Co-Director (Study director) - Aquatic toxicity on Fish
	Sang-Hee Shin	Project Co-Director (Study director) - Aquatic toxicity on Invertebrate
	Tae-Won Kim	Laboratory technician Field sampling manager
	Je-Kwan Park	Laboratory technician Field sampling technician
	Hyeong-Ju Seok	Laboratory technician Field sampling technician

Quality Assurance Project Plan (QAPP)

1 PROJECT AND TASK DESCRIPTION

1.1 Test site

Land-based test facility for BioViolet™ developed by Kwang San Co., Ltd. is located in Goseong-gun, Gyeongsangnam-do, Republic of Korea.

Table 3. System components of the BioViolet™

Components	Q'ty
Tank (250m^3)	3
Ballast pump	1
Flow meter	1
Pressure meter	2
Main control panel	1
Level transmitter	2
Level switch	3
Seawater pump	1
Sampling port	25
Sampling tank	3
Agitator	3
Piping & etc.	

Figure 3. Test site

1.2 The BioVioletTM BWMS

1.2.1 Ballasting mode

- Treated water

The test (challenge) water is transferred from tank 2 to tank 3 via filter and UV system by ballast pump. In this process, a flow meter is used to control the ballast pump for real-time maintenance of inside flow of main ballast pipe.

During ballasting operation, most aquatic organisms and any particles in the test water larger than 50 μm are strained through the filter. After filtration, aquatic organisms that are survived in the filtration process are effectively disinfected when passing through the UV system.

In Figure 4-1, red line presents the flow path of treated water in ballasting operation.

- Control water

The test (challenge) water is transferred from tank 1 to tank 2 by ballast pump. In this process, a flow meter is used to control the ballast pump for real-time maintenance of inside flow of main ballast pipe.

In Figure 4-2, blue line presents the flow path of control water in ballasting operation.

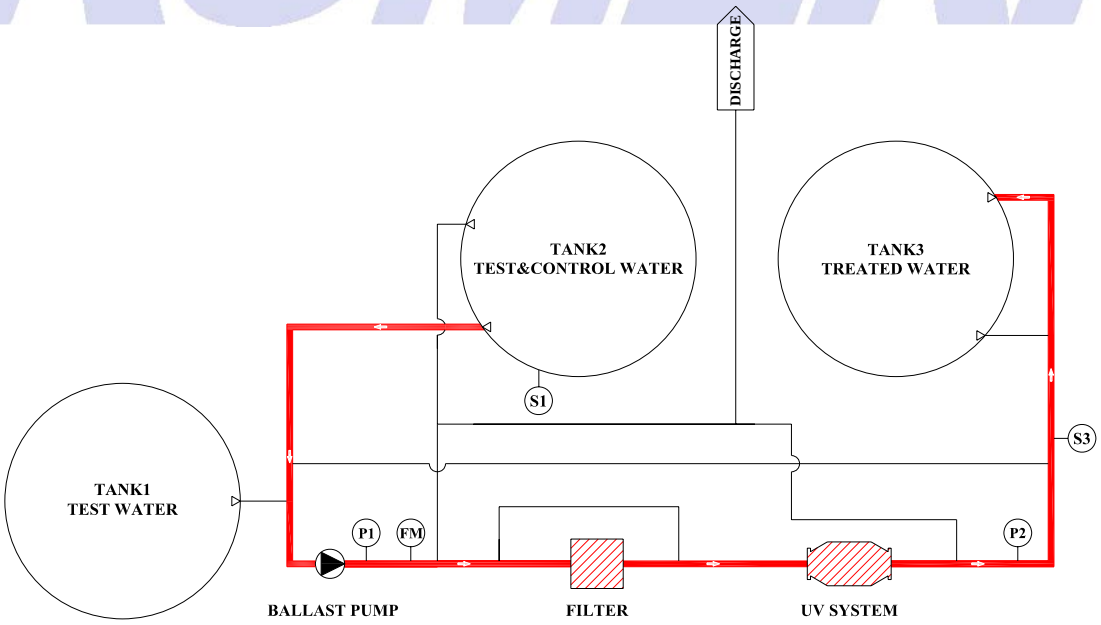


Figure 4-1. Treated water process at ballasting operation

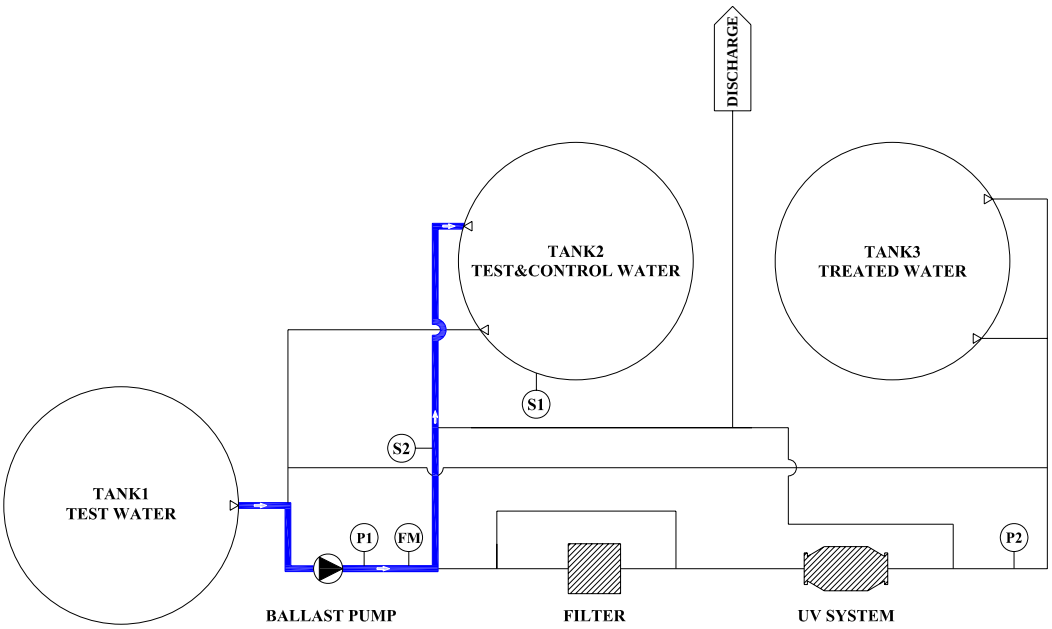


Figure 4-2. Control water process at ballasting operation

1.2.2 De-ballasting mode

- Treated water

The treated water which was stored for five days in tank 3 is discharged via the UV system by ballast pump.

The filter is not used while the treated water is discharged in de-ballasting operation. However, the UV system is used once more to disinfect aquatic organisms that are not disinfected in the ballasting process.

In this process, a flow meter is used to control the ballast pump for real-time maintenance of inside flow of main ballast pipe.

In Figure 4-3, red line presents the flow path of treated water in de-ballasting operation.

- Control water

The control water that was stored for five days in tank 2 is discharged by ballast pump.

In this process, a flow meter is used to control the ballast pump for real-time maintenance of inside flow of main ballast pipe.

In Figure 4-4, blue line presents the flow path of the control water in de-ballasting operation.

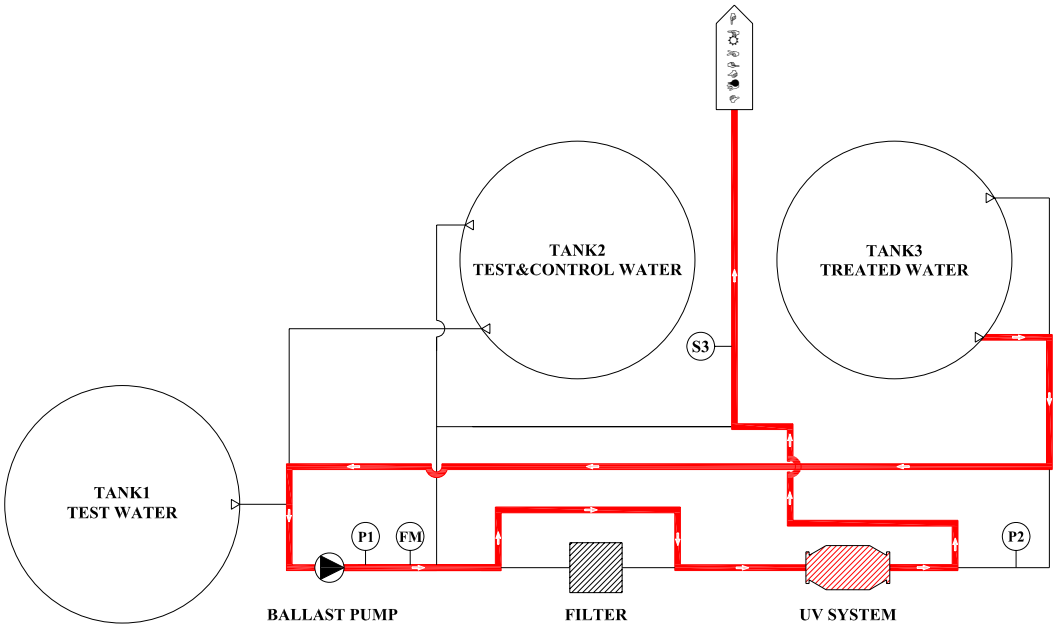


Figure 4-3. Treated water process at de-ballasting operation

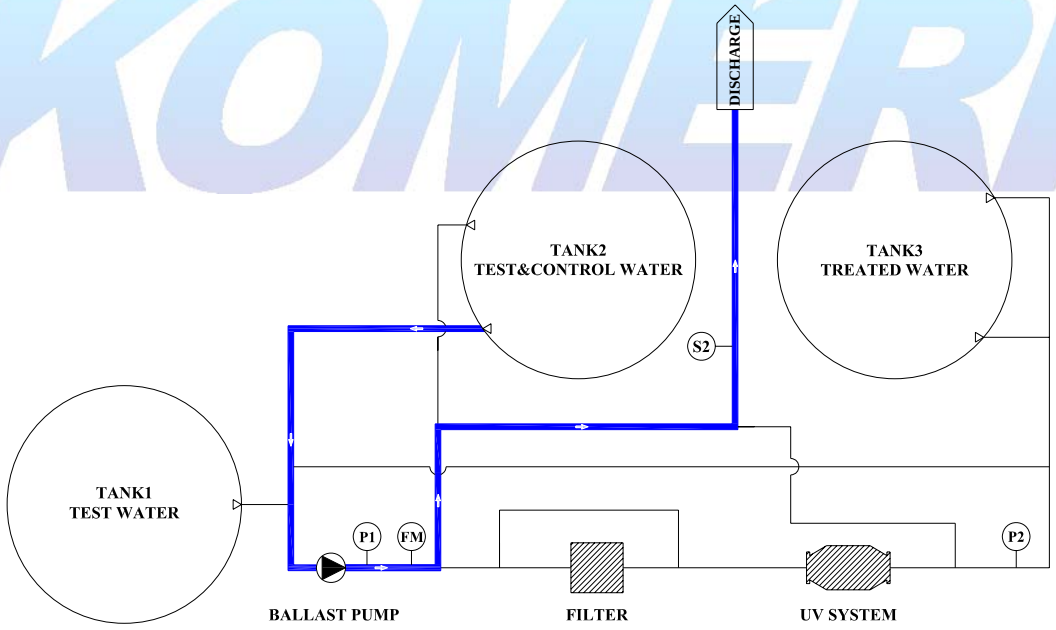


Figure 4-4. Control water process at de-ballasting operation

1.3 Task

The tests in accordance with the G8 and G9 of the BioVioletTM comprise three major task s:

1. Pre-test evaluation of system documentation.
2. Performance testing of the system, including collecting ballast water samples from land-based tests.
3. Analysis of samples collected from land-based tests.

This QAPP relates to Tasks 1, 2 and 3, which are the responsibility of the Korea Marine Equipment Research Institute (KOMERI). In addition, project co-actors, Korea Testing & Research Institute (KTR) and Marine Eco-Technology Institute Co., Ltd. (MEI) are engaged to support KOMERI in undertaking specific analyses, as follows:

KTR undertakes TOC/DOC analysis and chemical analysis of the collected samples. KTR is periodically participating in 'Inter-laboratory Comparison Test' to maintain quality assurance.

MEI undertakes aquatic toxicity test of the discharge (control and treated) water.

1.4 Test schedule and responsibility

Table 4. Test schedule and responsibility

Test		**Year / Month / Day**		**Responsibility**		
Period	Cycle	Ballasting	de-Ballasting	KOMERI[a]	KTR[b]	MEI[c]
> 32 PSU	1	2011/07/28	2011/08/02	○		
	2	2011/08/04	2011/08/09	○	○	○
	3	2011/08/11	2011/08/16	○		○
	4	2011/08/18	2011/08/23	○		
	5	2011/08/25	2011/08/30	○		
	6	2011/09/01	2011/09/06	○		
	7	2011/09/08	2011/09/13	○		
	8	2011/09/15	2011/09/20	○		
3 - 32 PSU	9	2011/09/22	2011/09/27	○		
	10	2011/09/29	2011/10/04	○	○	○
	11	2011/10/06	2011/10/11	○		○
	12	2011/10/13	2011/10/18	○		
	13	2011/10/20	2011/10/25	○		
	14	2011/10/27	2011/11/01	○		
	15	2011/11/03	2011/11/08	○		
	16	2011/11/10	2011/11/15	○		

[a] Efficacy test.
[b] Chemical analysis.
[c] Whole effluent toxicity test.

2 MEASUREMENT AND DATA ACQUISITION

2.1 Test process design

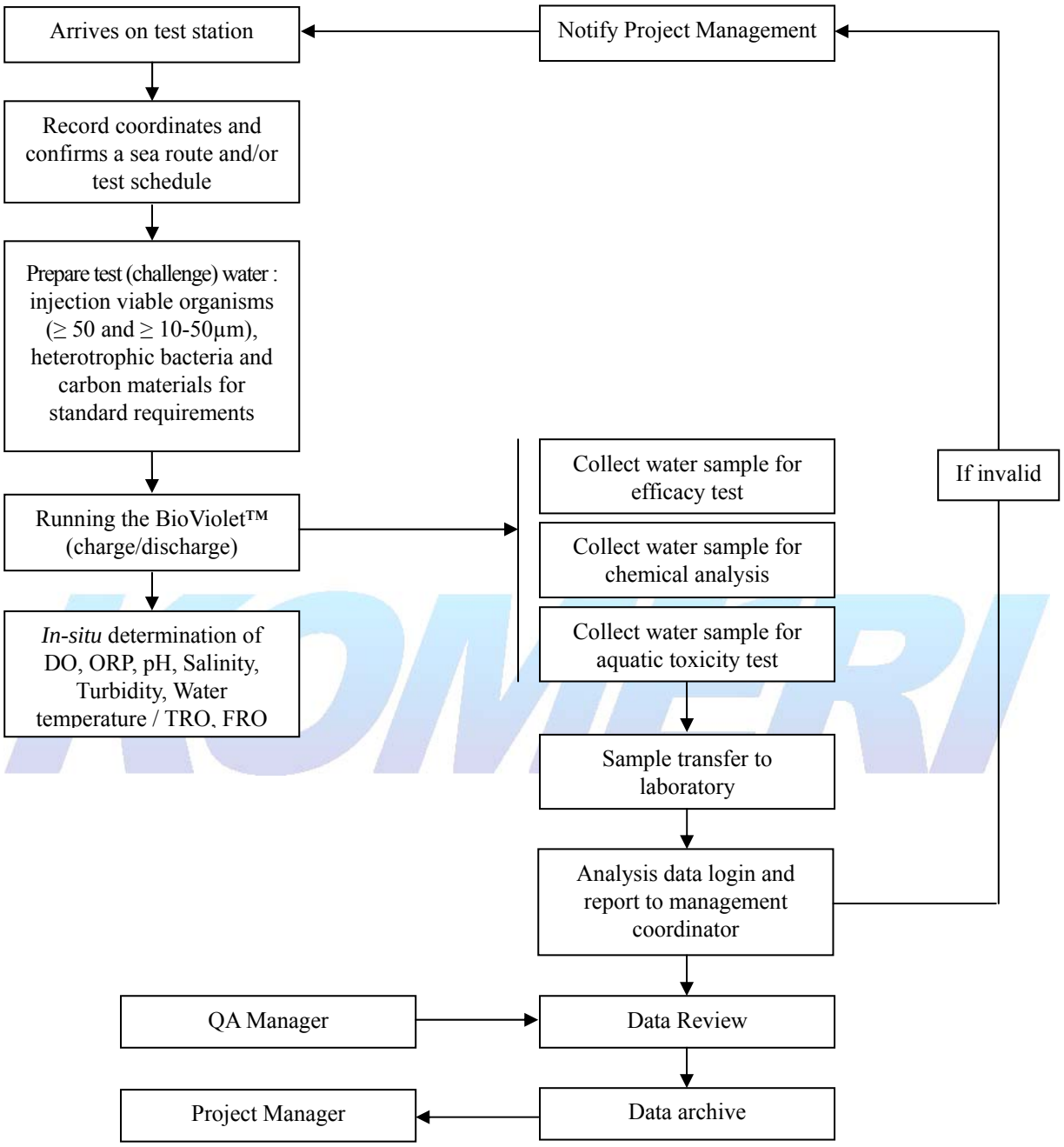


Figure 5. Test activities and data management process

2.2 Preparation of the test (challenge) water

2.2.1 Viable organisms

Various viable organisms are supplied for satisfying the condition of test (challenge) water in accordance with G8 (Table 5).

Table 5. Objective density, total volume and diversity of supplied organisms

Period	Cycle	≥ 50 μm organisms		≥ 10 - 50 μm organisms	
		Supplied organisms (inds./mL)	Total supplied vol. (L)	Supplied organisms (inds/mL)	Total supplied vol. (m^3)
> 32 PSU (Seawater)	1	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	2	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	3	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	4	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	5	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	6	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	7	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	8	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
3-32 PSU (Brackish water)	9	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	10	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	11	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	12	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	13	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	14	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	15	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4
	16	*Artemia* (5,000)	10	*Tetraselmis suecica* (250,000)	4

2.2.2 Dissolved organic carbon (DOC), particle organic carbon (POC) and total suspended solid (TSS)

Glucose, Starch and silica are supplied for satisfying the condition of DOC, POC and TSS in accordance with G8 (Table 6).

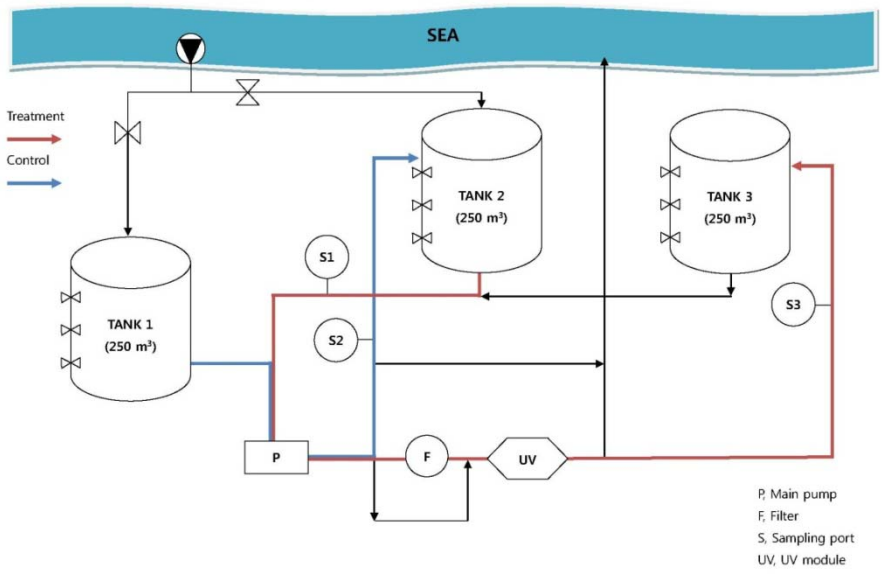
Table 6. Injection weight of soluble starch, glucose and silica supplied for DOC, POC and TSS

Period	Cycle	Starch (g)	Glucose (g)	Silica (kg)
> 32 PSU (Seawater)	1	1,250	625	0
	2	1,250	625	0
	3	1,250	625	0
	4	1,250	625	0
	5	1,250	625	0
	6	1,250	625	0
	7	1,250	625	0
	8	1,250	625	0
3-32 PSU (Brackish water)	9	6,000	3,000	10
	10	6,000	3,000	10
	11	6,000	3,000	10
	12	6,000	3,000	10
	13	6,000	3,000	10
	14	6,000	3,000	10
	15	6,000	3,000	10
	16	6,000	3,000	10

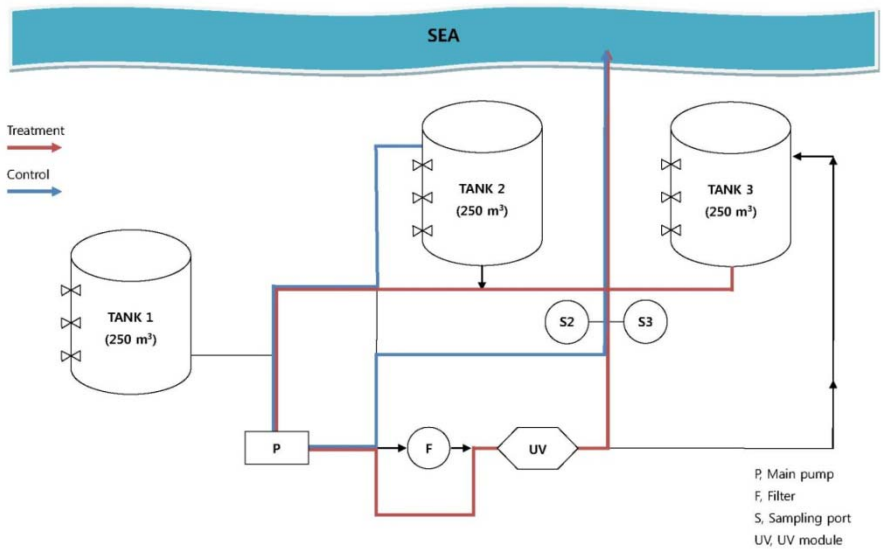
2.3 Sampling

Sample collection will be used during the sample collection periods. These plans will include detailed information on the sample locations, and the types of samples to be collected. The project manager will prepare the sample collection and brief the sample collection team on the objectives of the sampling.

2.3.1 Sampling information



(a) Ballasting mode



(b) De-ballasting mode

Figure 6. Sampling location

Table 7. Sample information

Sample	Sampling location	Volume of sample (L)		
		KOMERI	KTR	MEI
Test (challenge) water	S1	50	20	-
Treated water during ballasting on day 0	S2	150	50	-
Control water during ballasting on day 0	S3	50	20	-
Treated water during de-ballasting on day 5	S2	150	50	500
Control water during de-ballasting on day 5	S3	50	20	800

Table 8. Sample tag

Elapsed time (day)		Day 0	Day 1	Day 5
Test water (S1)		KSU-S1D0-1[a]	-	-
Control water (S2)	B	KSU -S2BD0-1	KSU -S2D1-1	KSU -S2BD5-1
	M	KSU -S2MD0-1		KSU -S2MD5-1
	E	KSU -S2ED0-1		KSU -S2ED5-1
Treated water (S3)	B	KSU -S3BD0-1	KSU -S3D1-1	KSU -S3BD5-1
	M	KSU -S3MD0-1		KSU -S3MD5-1
	E	KSU -S3ED0-1		KSU -S3ED5-1

[a] KSU-S1D0-1 – Sampling location 1 (S1) Day 0 (D0) – test cycle 1 (1)

2.3.2 Sampling methods

- Sample collection, preparation, decontamination procedure

Chemical water sampling will be performed according to Water quality-Sampling-Part 3: Guidance on the preservation (ISO 5667-3, 2003) and Coastal 2000 Northeast Component 'Field Operation Manual' (US EPA, 2000). Biological water sampling performed according to Standard Method of the Examination of Water and Wastewater (APHA, 2005).

- Sampling equipment, preservation, and holding times

Sampling for efficacy test

Viable organisms

≥ 50 μm organisms will be collected with 30 μm (diagonal size) plankton net immersed in a water tank and rinsed by filtered test water. Organisms were concentrated from 1 m^3 of treated water and 20 L of control water.

≥ 10-50 μm organisms will be collected with 10 μm (diagonal size) plankton net immersed in a water and rinsed the by filtered test water. Organisms were concentrated from 10 L of treated water and 1 L of control water.

All samples will be placed in a box with electronic temperature control adjusted to the ambient water temperature and transferred to the laboratory by car within 2 hours of sampling; sample analysis will commenced within an hour of arrival to the laboratory.

Bacteria

1 or 2 L sterilized disposable PE pack will be used for each sample collection. After sample collection, each sample bottle put into 4 °C cooler box. And then, transfer to laboratory by car within 2 hours. Sample analysis will launched within an hour after arrive sample to laboratory.

DOC/POC

Because of the possibility of oxidation or bacterial decomposition of some components of aqueous ampoules, the time between sample collection and the start of analysis should be minimized. Also, samples should be kept cool (4 ˚C) and protected from sunlight and atmospheric oxygen. In instances where analysis cannot be performed within 2 hr from time of sampling, the sample is acidified (pH < 2) with HCl or H_2SO_4 (7 days, ISO 5667-3).

Sampling for chemical analysis

VOCs/THMs

Collect the sample normally by immersion, by filling the bottle or the vial completely, discarding this water, refilling and stoppering so as to leave no headspace. Loss of volatile compounds through degassing of the sample should be avoided. Slowly fill the bottle at the sampling point until it overflows, avoiding turbulence. If reaction between free halogens and organic matter in the sample, to produce trihalogenated methanes, is to be eliminated, add an excess of sodium thiosulfate to the sampling bottle or vial after rinsing the bottle or the vial but prior to sampling (0.1-0.2 mL of 30 g/L solution).

AOX

Filter sample containing particles through a filtration apparatus with a pore width of 0.45 µm. Acidify to between pH 1 to pH 2 with HCl and cool to between - 1 ˚C to 5 ˚C, for 2 weeks.

Bromate

Sampling and storage of samples in glass bottles is preferable. Add 1.0 mL Ethylenediamine solution/1 L sample. If analysis cannot be performed within 2 hr from time of sampling, samples should be kept cool (4 ˚C).

HANs

Sampling and storage of samples in glass bottles is preferable. Add 10 mg ammonium chloride and 1-2 drop 6 N hydrochloric acid solution/1L sample. If analysis cannot be performed within 2 hr from time of sampling, samples should be kept cool (4 ˚C). The samples are stable for at least 14 days.

HAAs

Samples must be iced or refrigerated at 4 ˚C and maintained at these conditions away from light until extraction. Holding studies performed to date have suggested that, in samples preserved with NH_4Cl, the analytes are stable for up to 14 days. Extracts must be stored at 4 ˚C or less away from light in glass vials with Teflon-lined caps. Extracts must be analyzed within 7 days from extraction if stored at 4 ˚C or within 14 days if stored at -10 ˚C or less.

Halogenated phenols

Acidify to 2 mL H_2SO_4/1 L (pH < 2) sample and cool to between 1 ˚C to 5 ˚C, 2 days.

Sampling for whole effluent toxicity test

No special preparations are needed for this analysis on the field. Samples are transported in two kind of sampling water tank on a refrigerator car (constant 20-23 °C) to the MEI's laboratory. The test concentration is diluted on the discharge control water as crude liquid. The six test concentrations of test substance including 0.00 % (only discharge control water) and 100.00 % (only discharge treated water) are arranged for the final definitive experiment.

Sampling/measurement system failure response and corrective action process (see Figure 5).

2.3.3 Sample handling and custody

A procedure will be developed to collect, transport and store the samples for analysis that will minimize the possibility of contamination and introduction of artifacts. Special care will be taken to prevent the volatilization from filter sample, to prevent temperature damage of water sample, as well as to prevent contamination of collected samples from the ubiquitous gaseous air pollutants.

Specific procedures to ensure the integrity of the collected samples will be outlined in the SOPs developed for each instrument. However these should include the necessary procedures for ensuring sample validity by:

- Preparation of sampling material, including procedures to clean water samples, loading water samples into sampling apparatus, and transport of sampling media to field locations.
- Storage of sampling media once removed from sampling location including sealing procedures and temperature requirements for transportation from field locations to laboratory.
- Archiving of sampling material until the analyses can be performed including prevention of photochemical and temperature changes.
- Requirement for removing samples from archive for analyses that preserve sample integrity

Sample custody will be documented with sample log sheet for each water sample that will track the lifetime from preparation and cleaning, deployment to the laboratories, laboratory archiving until analysis, analysis and data reporting.
Samples are logged into the login notebook after arrival at the laboratory, and assigned a laboratory sample number.

2.4 Analytical methods

Accuracy of laboratory analyses will be assessed for compliance with the criteria established in Korea Laboratory Accreditation Scheme (KOLAS) for efficacy test and chemical analysis and appropriate system (OECD GLP system) for aquatic toxicity test. And all laboratory tests and analyses are undertaken by scientific and technical staff of KOMERI, KTR and MEI.

Table 9. Laboratory: Analytical methods

Parameter	**Method**
Basic water parameter	
Salinity	APHA Standard Method[a] 2520 B
Dissolved oxygen	ASTM D888-09 Test Method C
Temperature	APHA Standard Method 2550
pH	APHA Standard Method 4500-H^+ B
Oxidation-Reduction Potential	APHA Standard Method 2580
Total organic carbon	ISO 8245:1999

Dissolved organic carbon	ISO 8245:1999	
Total suspended solid	APHA Standard Method 2540 D	
Turbidity	APHA Standard Method 2130 B	
Efficacy test		
≥ 50 µm Organism	Fleming & Coughlan (1978)[b] US EPA 600/R-10/146 (2010)[c] APHA Standard Method 10200 C	
≥ 10-50 µm Organism	Anja *et al.* (2005)[d] APHA Standard Method 10200 C Manual and Guide, UNESCO (2005) UNESCO 4 (2003) EPA 445.0 (1997)	
Heterotrophic bacteria	APHA Standard Method 9215	
Total Coliform	APHA Standard Method 9222 B	
Escherichia coli	EPA 1603:2009	
Intestinal Enterococci	EPA 1600:2009	
Toxicogenic *Vibrio cholerae* (O1, O139)	APHA Standard Method 9260 H	
TOC (DOC/POC)	ISO 8245:1999	
Chemical analysis		
AOX	ISO 9562:2004	
Bromate	ISO 15061:2001	
Bromide/Chlorate	US EPA 300.1:1997	
VOCs/THMs	US EPA 524.2:1995	
Halogenated Phenols	US EPA 8041A:2007	
HAAs	EPA 552.2:1995	
HANs	EPA 551.1:1995	
TRO/FRO	ISO 7393-2:1985	
Density	ISO 15212-1:1998 Oscillation-type density meters-Part 1	
Aquatic toxicity test		
Growth inhibition test on algae	ISO 10253: 2006	
Acute/Chronic	Invertebrate	ASTM E1440-91: 2004 Janssen *et al.*, (1994)[e]
	Fish	OECD 203/OECD 212

[a] Standard Methods. 2012. In: A.D Eaton, L.S Clesceri, E.W Rice, A.E Greenberg (eds), *Standard Methods for the Examination of Water and Wastewater*. Baltimore, Maryland. APHA, AWWA and WEF.

[b] Fleming, J.M., Coughlan, J. 1978. Preservation of vitally stained zooplankton for live/dead sorting. Estuaries (1) 135-137.

[c] US EPA 600/R-10/146. 2010. Protocol for the Verification of Ballast Water Treatment Technologies. Section 5.4.6.4. p45.

[d] Anja S, T. Cheryl, S. James, S. Kristin. 2005. Application of Alamar blue/5-carboxyfluorescein diacetate acetoxymethyl ester as a noninvasive cell viability assay in primary hepatocytes from rainbow trout. *Analytical Biochemistry*. (344) 76-85.

[e] Janssen, C.R., G. Persoone and T.W. Snell. 1994. Cyst-based toxicity tests. VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*. Aquatic Toxicology, (28) 243-258.

2.5 Data acquisition

2.5.1 Documentation and records

- QAPP

The Master Copy of the QAPP will be kept in electronic copy at a security data server at KOMERI.

The Quality Assurance Manager, Jae-Uk Kang, is the overall Quality Manager for this QAPP and has responsibility for controlling its currency and ensuring that all personnel listed in Approval Sheet have up-to-date, controlled copies of the document, sent by email as a Read Only (non-changeable) PDF file. A Record of Distribution is to be kept as hard copy on file no. [Data file for BWMS test BioViolet™ (TYPE APPROVAL)] and saved in electronic copy at file directory [D:\BWMS\ BioViolet\type\KOMERI\QAPP].

All personnel listed in Approval Sheet are to confirm by email to the Project/Quality Manager when they receive the QAPP, including any updated versions. Such confirmation e-mails are to be printed and filed as hard copy on file no. [Data file for BWMS test BioViolet™ (TYPE APPROVAL)] and saved in electronic copy at file directory [D:\BWMS\ BioViolet\type\KOMERI\QAPP].

Weekly and/or Monthly Project Progress Meetings of the team leaders of each group involved in the G9 testing are to be held at the KOMERI and any suggested changes/updates to the QAPP made at those meetings – which are to be fully minuted.

The Quality Manager will then ensure that the updated QAPP is distributed to all personnel listed in Approval Sheet, again by email as a Read Only (non-changeable) PDF file, and that the Record of Distribution is completed. Personnel are to be confirmed by email to the Project/Quality Manager (jukang@komeri.re.kr) when they receive updated versions of the QAPP, which are to be filed as per paragraph.

The QAPP Document Control Process is shown Figure 7:

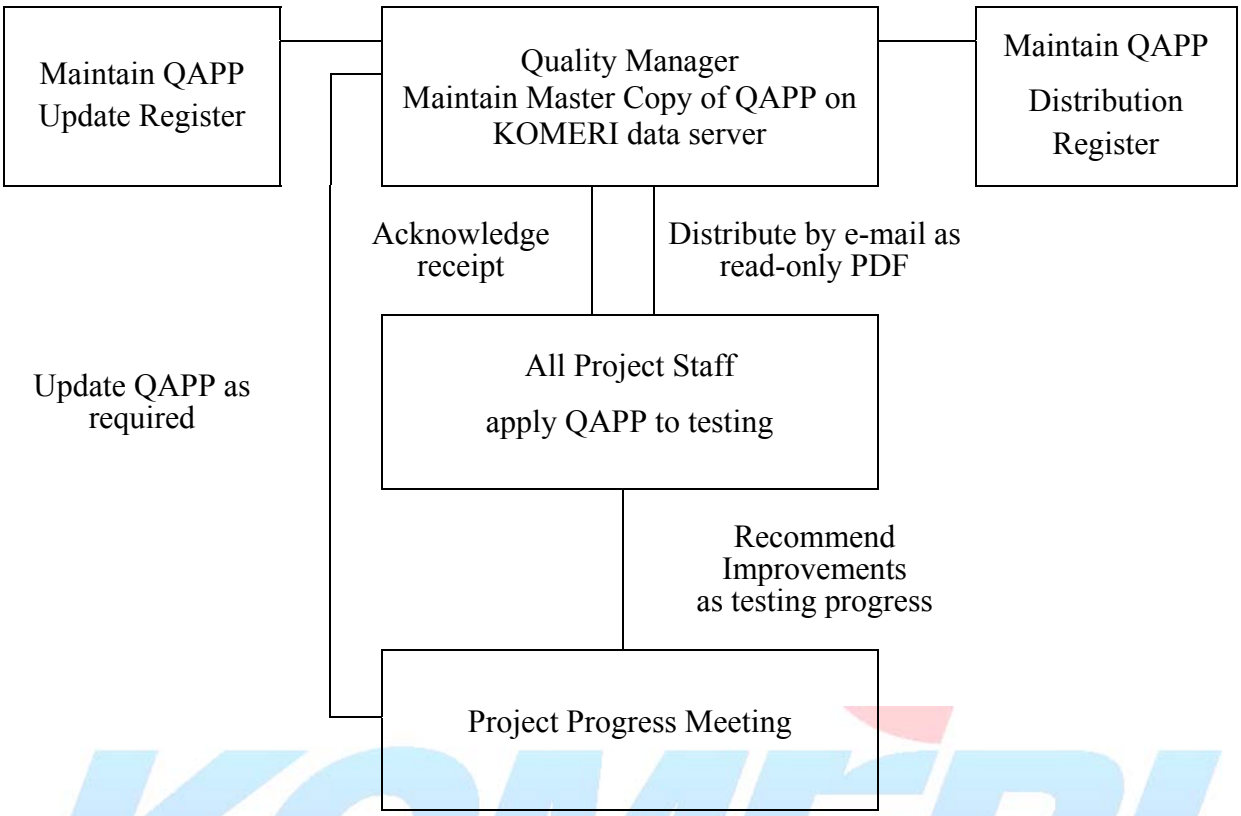


Figure 7. QAPP Document Control Process

- Field sample records

Field sample records are to be accompanied by a Chain of Custody (COC) format at each sampling time, and then binding for keeping.

- Chain of custody (COC) records

The transport of all samples from field sampling to the laboratory for analysis is to be accompanied by a COC. The COC is to be completed at each step in the transport chain, with each party in the transport exchange keeping a copy of the Record; which is to be filed by each party for future reference if necessary.

- Laboratory raw data records

Analysis raw data log sheets are used for data recording and are to be accompanied by a COC format at each time of analysis, and then binding for keeping.

2. 5. 2 Data management

- Data recording

Data are transposed from field notebooks to an electronic database, and from laboratory reports to an electronic database.

- Data validation

Data are validated to insure that the system performs the intended function consistently, reliable, and accurately in generating the data in accordance with KOLAS and appropriate system (OECD GLP system).

- Data transformation

It is expected that data transformations made during this investigation will be relatively simplistic and all calculations made during data transformation will be checked 100% prior to dissemination of the transformed information.

- Data transmittal

During the transfer of data from one place (field notebook or data report) to another (electronic data spreadsheet) the data will be copied and checked by one individual and then checked 100% by a second individual to insure accuracy.

- Data reduction

Raw data from field measurements are recorded directly in field notebooks or on sample log. If errors are made, results will be legibly crossed out, legibly signed and dated by the person recording the data, and corrected in a space adjacent to the original entry. Logbooks will be periodically reviewed by the Project Director and Quality Assurance Manager to insure that records are complete, accurate, and legible. Reduction of current water quality test data will be made by entering all field collected data in an EXCEL® computer spreadsheet.

Laboratory data reduction procedures will be performed according to the following protocol.

All information related to analyses will be documented in controlled laboratory logbooks, instrument printouts, or other approved forms. All entries that are not generated by an automated data system will be made neatly and legibly in waterproof ink. Corrections will be made by drawing a single red line through the error and entering the correct information adjacent to the cross out. All changes will be legibly signed, dated, and if appropriate, accompanied by a brief explanation. Analytical laboratory records will be reviewed by the

Section Supervisors on a regular basis and by the laboratory QA/QC Officer periodically, to verify adherences to documentation requirements.

- Data analyses

The data generated during initial test periods in this project will be used to calculate the efficiency of ballast water treatment system during the test periods. Treatment efficiency will be used to calculate charging/discharging to and from the ballast tank, and to verify the system durability and/or stability.

- Data tracking

Data will be recorded in the field notebooks and upon return completion of the associated data collection information will be transposed to an electronic spreadsheet format. Copies of field data will be made and stored in project file on a daily basis. Laboratory data will also be transposed to an electronic spreadsheet format upon receipt.

- Data storage and retrieval

Data will be maintained in electronic format using MS EXCEL for data analyses and presentation purposes. Backup copies of all data files will be made intermittently throughout the project and upon completion of the project, a DVD containing all electronic data will be produced with copies available for distribution.

- Data release

Prior to being released as final, laboratory data will proceed through a tiered review process. Each analyst will be responsible for reviewing the analytical and QC data that he/she has generated. As part of this review, the analyst will verify that:

- The appropriate methodology was used,
- Instrumentation was functioning properly,
- QC analyses were performed at the proper frequency and the analyses met the acceptance criteria,
- Samples were analyzed within due time,
- All data were generated within the calibration range,
- Matrix interference problems were confirmed,
- Method-specific analytical requirements were met, and
- Calculations, dilution factors, and detection limits were verified.

Prior to releasing the final data, the section supervisor will review the data to:

- Verify the appropriate methodology was used,

- Verify QC analyses were performed at the proper frequency and the analyses met the acceptance criteria,
- Verify samples were analyzed within due time,
- Review and document problems encountered during the analyses.

The final data report will be reviewed and approved by laboratory QA/QC Officer and the laboratory project manager prior to its release.

2. 5. 3 Data validation and usability

- Data review, validation, and verification

.1 Data review

Once these goals and objectives are evaluated and approved the BioViolet™, analytical data quality will be assessed to determine if the objectives have been met. In addition, the data will be reviewed by KOMERI's quality assurance officer for indications of interference of results by sample matrices, cross contamination during sampling, cross contamination in the laboratory, and sample preservation and storage anomalies.

.2 Validation and verification methods

Efficacy test

The procedures used to evaluate field and laboratory analyses data will include checking procedures used in the field, ensuring that field measurement equipment was properly calibrated, checking for transcription errors, and comparing the data to historic data or verifying its 'reasonableness'. Evaluation of field data will be the responsibility of the Project Manager or his designed.

An independent assessment of the data will be performed by KOMERI. The overall completeness of the data package will be evaluated. Completeness checks will be administrated on all data to determine whether the deliverables in accordance with the requirements specified in the QAPP are present.

e.g, Accuracy of laboratory analyses will be assessed for compliance with the criteria established in section Calibration and Quality Control Check of the Final Report using the analytical results of method blanks and DOC/POC samples. The percent recovery (%R) for DOC/POC samples will be determined according to the following equation:

$$\%R = \frac{(Amount\ in\ spiked\ sample \times Amount\ in\ sample)}{Known\ amount\ added} \times 100$$

%R for DOC/POC will be determined according to the following equation:

$$\%R = \frac{Experimental\ concentration}{Known\ amount\ added} \times 100$$

In case of viable organisms (≥ 50 and ≥ 10-50 μm) that cannot be identified in the samples are to be sent to an appropriate agency for identification by an expert taxonomist where possible. It might be noted here that smaller phytoplankton are poorly known taxonomically and it may not be possible to identify these. Data from expert taxonomists are compared with archived KOMERI data for confirmation.

Precision of quantitative methods (APHA Standard Methods 9020B) will be used for data assessment of heterotrophic bacteria.

- Perform duplicate analyses on the first 2 positive samples of each type, with each set of duplicates analyzed by a single analyst. Duplicate analyses are recorded as D_1 and D_2.
- Calculate the logarithm of each result. If either of a set of duplicate results is <1, add 1 to both values before calculating the logarithms.
- Calculate the range (R) for each pair of transformed duplicates as the mean ($\overline{R}$) of these ranges.

Thereafter, analyze 10 % of routine samples in duplicate

Chemical analysis

Test methods applied ISO, ASTM, Standard method, OECD Guideline. If KTR develops or introduces new test methods for the autonomous use, validation and verification of test methods should be conducted according to KTR QP-17.

3 QUALITY ASSURANCE SYSTEM

3.1 Quality assurance objects

3.1.1 Efficacy test

Quality assurance objectives for the KOMERI listed in Table 10. Individual research/study/test projects may develop QA objectives that will supersede the objectives listed in the Table 10.

Table 10. Quality assurance objectives of KOMERI

Analyte	**Unit**	**Method Detection Limit**	**Concentration Range**	**Precision Objective (Resolution)**	**Accuracy Objective**
Temperature	°C	NA	-5~50	0.01	± 0.1
Salinity	PSU	1.0	≤ 100	1%	± 1.0
pH	pH unit	0.0	0-14	± 0.01	± 0.2
Dissolved oxygen	mg/L	0.1 0.2	< 8 mg/L > 8 mg/L	0.01	± 0.1 ± 0.2
ORP	mV	1.0	-999 - 999	1.0	± 20
Turbidity	NTU	0.1	0-100 NTU 100-400 NTU 400-3000 NTU	0.1 - 1.0	1% 3% 5%
Dissolved organic carbon	mg C/L	0.36	0-25 000 mg/L	2.5 %	CV 1.5 % Max.
Total organic carbon[a]	mg C/L	0.36	0-25 000 mg/L	2.5 %	0.02
Total suspended solid	mg/L	0.1	NA	5%	±0.10
Organisms, ≥ 50 µm	ind./mL	1.0	NA	NA	±1.0
Organisms, ≥10~50 µm	ind./mL	1.0	NA	NA	±1.0
Heterotrophic bacteria	cell/mL	1.0	30-300	5%	±1.0
Total Coliform	CFU/100 mL	1.0	20-80	5%	±1.0
Escherichia coli	CFU/100 mL	1.0	20-80	5%	±1.0
Intestinal Enterococci	CFU/100 mL	1.0	20-60	5%	±1.0
Vibrio cholerae (serotype O1 and O139)	CFU/100 mL	1.0	NA	10%	±1.0

[a] Quality Control of TOC maintained by inter-laboratory comparison test.

Each test day, water quality meters (multi-probe) will be pre-calibrated prior to the commencement of field activities in accordance with manufacturer's instruction. Suspect calibration information will be highlighted in the field data notebook upon discovery of the information. Data collected during the period of suspect information will be footnoted as being questionable.

Table 11. Quality assurance sample types, frequency of use, and types of data generated for KOMERI

Parameters	**QA sample type or measurement procedure**	**Frequency of use**	**Data generated for calibration and redundancy**
Chemical water quality MS5 sonde (Hydrolab)			
Salinity	Certified standard conductivity solution (~50 µS/cm)	Each test cycle	Difference between probe value and standard level
Dissolved oxygen	Water-saturated air calibration	Each test cycle	Difference between probe value and saturation level
Temperature	QC check with standard thermometer	Each test cycle	Difference between probe and thermometer
pH	QC check with standard buffers	Each test cycle	Difference between probe and standards
Dissolved organic carbon	QC check with standard (potassium hydrogen phthalate) / Proficiency testing	Every 10 samples / Annually	Difference between sample and duplicate
Total organic carbon	QC check with standard (potassium hydrogen phthalate) / Proficiency testing	Every 10 samples / Annually	Difference between sample and duplicate
Total suspended solid	QC check with duplicate	Each test period	Difference between sample and duplicate
Chemical water quality Turbidity meter (HACH)			
Turbidity	Certified standard turbidity solution (0, 10, 100, 1000 NTU)	Each test period	Difference between sample and duplicate
Biological water quality			
Organisms ≥ 50 µm	QC check duplicate and other identification data	Each test period / species assemblage	Comparison of 5 samples, standard deviation
Organisms ≥ 10-50 µm	QC check duplicate and other identification data	Each test period / species assemblage	Comparison of 5 samples, standard deviation
Bacteriological water quality			
Heterotrophic bacteria	QC check duplicate	Each test period and/or every sample	Comparison of 5 samples, standard deviation
Total Coliform	QC check duplicate	Each test period and/or every sample	Comparison of 5 samples, standard deviation
Escherichia coli	QC check duplicate	Each test period and/or every sample	Comparison of 5 samples, standard deviation
Intestinal Enterococci	QC check duplicate	Each test period and/or every sample	Comparison of 5 samples, standard deviation
Toxicogenic *Vibrio Cholerae* (O1, O139)	QC check duplicate	Each test period and/or every sample	Comparison of 5 samples, standard deviation

3.1.2 Chemical analysis

Quality assurance objectives & Procedure for the KTR Laboratory listed in Figure 8 and Table 11. Individual research/study/test projects may develop QA objectives that will supersede the objectives listed here. It is objected to validate results by writing SOPs to assure the reliability of the test results. QA/QC aim to report all analytical procedures from sampling to results and validation factors during analysis procedure are corresponding

linearity and range, accuracy and precision, and the detection limit and quantitation limit. Given data generally followed specified test methods.

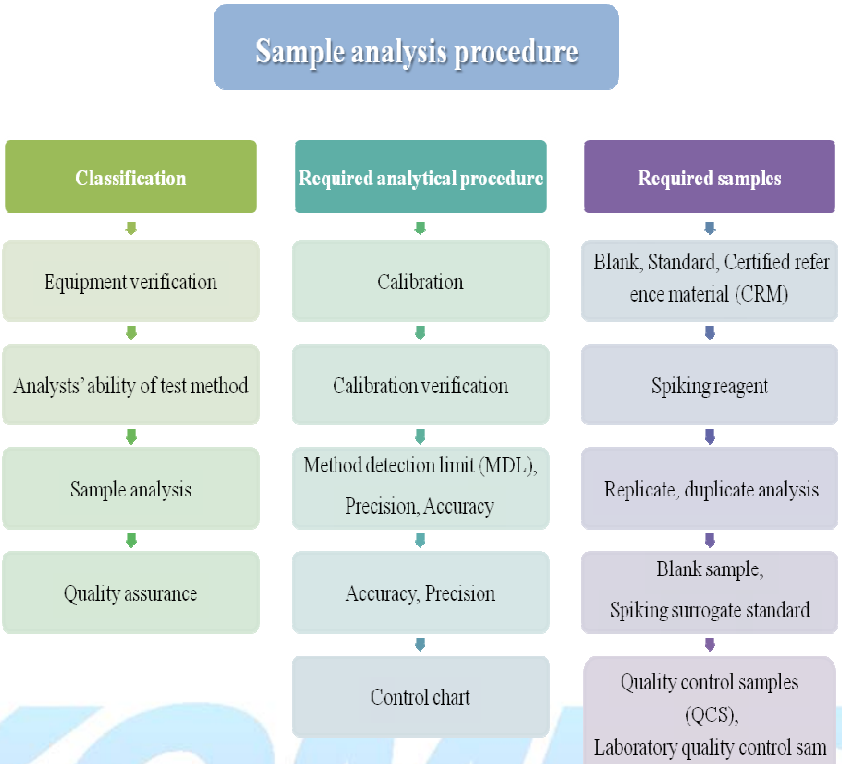


Figure 8. Procedure for quality assurance of analysis result

Table 12. Quality assurance objectives of KTR

Analysis item	unit	Method Detection Limit[a]	Concentration Range[b]	Precision Objective[c]	Accuracy Objective[d]
TRO	mg/L	0.03	0-10.0	NA[e]	NA
FRO	mg/L	0.03	0-10.0	NA	NA
Ozone(O_3)	mg/L	0.13	NA	NA	NA
ClO_2	mg/L	0.02	NA	NA	NA
Sulfide(S^{-2})	mg/L	0.02	NA	NA	NA
Bromate (BrO_3^-)	μg/L	0.08	5-100	0.56	105
Bromide(Br^-)	mg/L	0.01	1-10.0	0.34	96.0
Chlorate(ClO_3)	mg/L	0.09	1-10.0	0.45	94.0
AOX	mg/L	-	NA	NA	NA
DOC	mg/L	0.08	0-10.0	0.3	103
POC	mg/L	0.08	0-10.0	0.3	103
1,1-Dichloroethene	μg/L	0.02	1-500	0.2	100
Dichloromethane	μg/L	0.02	1-500	0.2	100
trans-1,2-Dichloroethene	μg/L	0.01	1-500	0.1	100
1,1-Dichloroethane	μg/L	0.01	1-500	0.1	100
cis-1,2-Dichloroethene	μg/L	0.01	1-500	0.1	100
Bromochloromethane	μg/L	0.01	1-500	0.2	100

Trichloromethane	μg/L	0.01	1-500	0.1	100
1,2-Dichloroethane	μg/L	0.01	1-500	0.2	100
1,1,1-Trichloroethane	μg/L	0.01	1-500	0.2	100
Tetrachloromethane	μg/L	0.01	1-500	0.2	100
Dibromomethane	μg/L	0.05	1-500	0.2	100
1,2-Dichloropropane	μg/L	0.02	1-500	0.2	101
Dichlorobromomethane	μg/L	0.02	1-500	0.2	100
1,1,2-Trichloroethane	μg/L	0.01	1-500	0.1	100
Dibromochloromethane	μg/L	0.01	1-500	0.2	100
Tetrachloroethene	μg/L	0.01	1-500	0.2	100
1,1,1,2-Tetrachloroethane	μg/L	0.01	1-500	0.1	100
1,2,3-Trichloropropane	μg/L	0.02	1-500	0.3	101
Chlorobenzene	μg/L	0.01	1-500	0.3	100
Tribromomethane	μg/L	0.03	1-500	0.1	101
1,1,2,2-Tetrachloroethane	μg/L	0.02	1-500	0.2	100
Bromobenzene	μg/L	0.01	1-500	0.2	100
2-Chlorotoluene	μg/L	0.02	1-500	0.1	100
4-Chlorotoluene	μg/L	0.02	1-500	0.3	100
1,2-Dibromo-3-chloropropane	μg/L	0.02	1-500	0.2	100
1,2,4-Trichlorobenzene	μg/L	0.02	1-500	0.3	101
1,2,3-Trichlorobenzene	μg/L	0.02	1-500	0.3	100
1,3,5-Tribromobenzene	μg/L	0.04	1-500	0.3	101
1,2,4-Tribromobenzene	μg/L	0.04	1-500	0.3	101
Trichloroacetonitrile	μg/L	0.01	10-5000	0.8	97.9
Dichloroacetonitrile	μg/L	0.01	10-5000	12.7	112
Chloral hydrate	μg/L	0.01	10-5000	3.5	100
Chloropicrin	μg/L	0.01	10-5000	1.7	95.9
Bromochloroacetonitrile	μg/L	0.01	10-5000	1.5	101
Dibromoacetonitrile	μg/L	0.01	10-5000	1.6	102
Monochloroacetic acid	μg/L	0.24	6-600	2.1	103
Monobromoacetic acid	μg/L	0.04	4-400	0.9	96.5
Dichloroacetic acid	μg/L	0.02	6-600	0.5	92.5
Dalapon	μg/L	0.04	4-400	0.8	96.7
Trichloroacetic acid	μg/L	0.02	2-200	1.6	90.9
Bromochloroacetic acid	μg/L	0.02	4-400	4.1	89.0
Dibromoacetic acid	μg/L	0.01	2-200	1.1	90.7
Bromodichloroacetic acid	μg/L	0.03	4-400	2.9	88.5
Tribromoacetic acid	μg/L	0.24	20-2000	3.9	96.5
2-Chlorophenol	μg/L	0.04	0.50-50.0	4.7	89.9
3-Chlorophenol	μg/L	0.04	0.50-50.0	6.7	93.7
4-Chlorophenol	μg/L	0.04	0.50-50.0	7.0	97.0
2,6-Dichlorophenol	μg/L	0.07	0.50-50.0	4.4	89.0
2,5-Dichlorophenol	μg/L	0.09	0.50-50.0	6.0	90.6
2,4-Dichlorophenol	μg/L	0.05	0.50-50.0	6.0	94.3
3,5-Dichlorophenol	μg/L	0.03	0.50-50.0	6.1	93.2
2,3-Dichlorophenol	μg/L	0.07	0.50-50.0	6.6	93.3
3,4-Dichlorophenol	μg/L	0.03	0.50-50.0	7.0	98.2
2,4,6-Trichlorophenol	μg/L	0.04	0.50-50.0	6.2	91.4
2,3,6-Trichlorophenol	μg/L	0.03	0.50-50.0	7.5	90.5
2,4,5-Trichlorophenol	μg/L	0.04	0.50-50.0	7.0	93.0

2,3,5-Trichlorophenol	μg/L	0.03	0.50-50.0	6.9	94.1
3,4,5-Trichlorophenol	μg/L	0.15	0.50-50.0	6.5	109
2,3,4-Trichlorophenol	μg/L	0.03	0.50-50.0	5.0	93.4
2,3,5,6-Tetrachlorophenol	μg/L	0.05	0.50-50.0	12.6	91.1
2,3,4,6-Tetrachlorophenol	μg/L	0.10	0.50-50.0	11.9	90.1
2,3,4,5-Tetrachlorophenol	μg/L	0.12	0.50-50.0	10.8	93.5
Pentachlorophenol	μg/L	0.06	0.50-50.0	5.5	96.5
2,6-Dibromophenol	μg/L	0.04	0.50-50.0	6.1	87.0
2,4-Dibromophenol	μg/L	0.10	0.50-50.0	8.2	95.9
2,4,6-Tribromphenol	μg/L	0.13	0.50-50.0	12.7	93.8
Hydrogen gas	%	0.05	0.05-100	NA	NA
Ozone gas	μL/L	0.05	0.2-2000	NA	NA
Cl_2 gas	μL/L	0.1	0.5-4000	NA	NA
O_2 gas	%	0.05	0.05-100	NA	NA
Density	g/cm^3	0.0001	0.8-1.3	NA	NA

[a] The method detection limit is determined as a one-sided 99% confidence interval from repeated measurements of a lowest-level standard across several calibration curves, and as a data from manufacturer or Detection limit was calculated value that S × 3.14

[b] Concentration Range =

- Initial calibration should be conducted with at least five different concentration of target analytical standard (eg. 1, 5, 10, 20, 40)
- If response factors or correlation factors are used, relative standard deviation (RSD) of each analyte should be ≤ 20%
- If the linear regression method is used, correlation coefficient should be > 0.99

[c] Precision is estimated as the percent relative standard deviation of repeated measurement (7 times) at the low constant concentration.

Precision (%) = RSD =S/x × 100 ; x : Mean measured value, S : standard deviation

[d] Accuracy is estimated as the difference between the measured and target values of performance evaluation samples at the lower concentration range and as the difference at the higher concentration range.

Accuracy (%) = $x/x_i \times 100$; x_i : Certified or theoretical value, x : Mean measured value

[e] NA : Not available.

If necessary, each test day, water quality meters will be pre-calibrated prior to the commencement of field activities in accordance with manufacture' instruction.

3.1.3 Aquatic toxicity

Quality assurance objectives and procedure for the MEI Laboratory show on Table 11 and Figure 9. Individual research/study/test projects may develop QA objectives that will supersede the objectives listed here. It is objected to validate results by writing SOPs to assure the reliability of the test results. QA/QC aims to report all aquatic toxicity test procedures from study plan to final report.

Table 13. Quality assurance objectives of MEI

Divisioin	**Test organism**	**Reference substance**	**Precision object**	**Criteria of Validation**
Algae	Diatom (*Skeletonema costatum*)	Potassium dichromate	72hr-EC50: 2.5±1.1 mg/L[a]	- Variation coefficient of the control specific growth rate: ≤ 10% - Specific growth rate: ≥ 0.92 day^{-1}
Invertebrate	Rotifer (*Brachionus plicatilis*)	Copper	24hr-LC50: 80 μg/L[b] (95% confidence limits: 30-130 μg /L)	- ≥ 90 % survival of all control organisms

Fish	Flounder fish (*Paralichthys olivaceus*)	NA[c]	NA	- Acute: ≥90 % survival of all control organisms
				- Chronic: Water temp. change: <±1.5 ℃ Survival of fertilized eggs in the controls: > 70 %

[a] Precision object of algae is applied by inter-laboratory test results in ISO10253 (2006) and ASTM E1440-91(2004).
[b] Precision object of rotifer is applied by inter-laboratory test results in ISO10253 (2006) and ASTM E1440-91(2004).
[c] NA: not available data

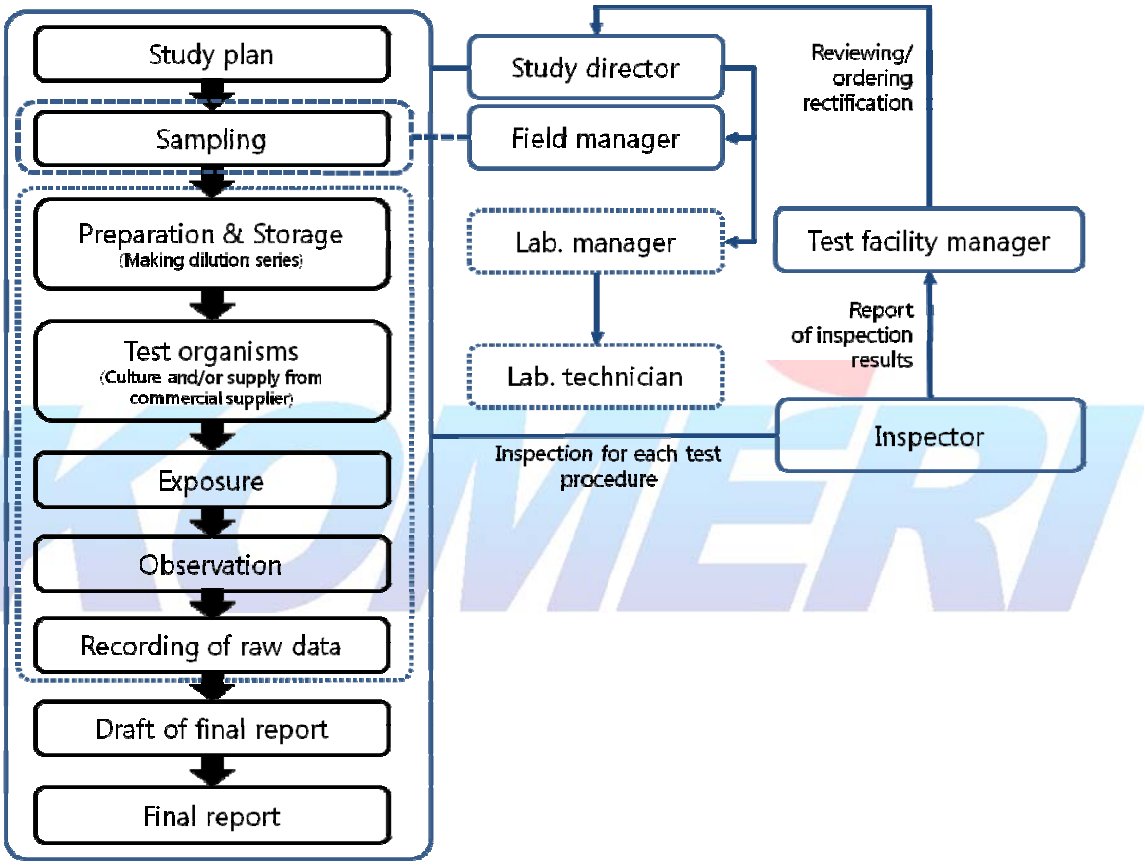


Figure 9. Flow chart for quality control and assurance of aquatic toxicity test (black rounding box: each test step; blue rounding box: responsible persons).

3.2 Quality control procedure

Three types of internal quality control samples and duplicates are routinely used at the laboratories. These samples are summarized and described here. Miscellaneous quality control checks are described below.

A field duplicate is a sample which is collected immediately after the regular sample at the same site. This type of co-located field duplicate estimates precision of the whole sampling process including inherent variability at the field site.
The quality control samples used are:

- A synthetic water quality control check sample is analyzed at least twice in each run of pH, salinity, temperature, oxidation-reduction potential, dissolved oxygen and turbidity samples.

4 ASSESSEMENT/OVERSIGHT

4.1 Assessments and corrective actions

The laboratory as part of their QA program will conduct laboratory performance and system audits. System audits will be done on an annual basis at a minimum and will include an examination of laboratory documentation on sample receipt/log-in/storage/chain-of-custody, procedures, sample preparation and analyses, instrument operating records, etc.

Field audits will include examination of field sampling records/screening results/instrument operating records, sample collection, handling, and packaging in compliance with the established procedures, maintenance of QA procedures, chain-of-custody, etc. Follow-up audits will be conducted to correct deficiencies, and to verify that QA procedures are maintained through the investigation. The audits will involve reviews of field measurement records, instrumentation calibration records, and sample documentation.

Corrective action is the process of identifying, recommending, approving, and implementing measures to counteract unacceptable procedures or out-of-limit QC performance that can affect data quality. Corrective action can occur during field activities, laboratory analyses, data validation, and data assessment. Corrective action should only be implemented after approval by the Project Manager, or his designee.

For non-compliance problems, a formal corrective action programme will be determined and implemented at the time the problem is identified. The person who identifies the problem is responsible for notifying the Project Manager.

4.2 Reports to management

The results of assessments conducted under this QAPP will be reported to management during the weekly (after 1 period test) Project Progress Meeting and remedial action taken in accordance with the procedures outlined in section 2.5.

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